Affinity purification of MHC class I and II molecules from the infected DC2.4 cells

1. Wash the infected DC2.4 cells twice in 5 ml cold PBS.
2. Lyse 10^7 DC2.4 cells with 1 mL lysis bufer for 45 min at 4 ^oC on a rotator.
3. Collect the SUPERNATANT after 20 min centrifugation at 20,000g, 4 ^oC.
4. Mix the supernatant with 5 µL antibody (500 µg: 1 µg)(GTX80040_Rat IgG2b_anti-Mouse-MHCII) and incubate at 4 ^oC on a rotator overnight.
5. Wash magnetic beads (Protein G) with CHAPS buffer (500 µg: 15 µL beads).
6. Incubate the sample with beads at 4 ^oC on a rotator for 2 hr.
7. Wash the sample-binding beads 4 times with CHAPS buffer.
8. Add SDS sample buffer and boil at 95 ^oC for 4 min.

Western blot analysis to evaluate the yield of immunoprecipitated MHC class I and II-peptides complex

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